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IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF	:
KEJUN FAN	: EXAMINER: KIM, YOUNG J
SERIAL NO: 10/627,780	:
FILED: JULY 28, 2003	: GROUP ART UNIT: 1637
FOR: NUCLEIC ACID-SEPARATING METHOD AND NUCLEIC ACID-EXTRACTING REAGENT	:

DECLARATION UNDER 37 C.F.R. 1.132

COMMISSIONER FOR PATENTS
ALEXANDRIA, VIRGINIA 22313

SIR:

I, Dr. Kejun Fan hereby declare:

1. I am a named inventor on the above-identified application.

2. I was born on January 17, 1961 in Hangzhou, CHINA.

3. In March, 1985, I graduated from Yokohama National University, Faculty of Engineering, majoring in material chemistry

4. In March, 1987, I received Master's degree from Graduate School of Yokohama National University, Faculty of Engineering, majoring in material chemistry (biochemistry, protein engineering).

5. In March, 1990, I Received Ph. D. from Graduate School of Yokohama National University, Faculty of Engineering, majoring in material chemistry (biochemistry, protein engineering).

6. From April, 1990 to the present, I have been employed by JSR Corporation, the assignee of this application, and I am engaged in research and development in the fields of application of polymer microparticles into clinical diagnosis, antigen—antibody interactions, protein purification, evaluation of protein function, nucleic acid purification, applications of nucleic acids, gene engineering, applications to genetic diagnosis, studies of colloid chemistry, synthesis and evaluation of particles, and related areas.

7. The following experiments were performed by me or under my supervision and control.

8. These experiments show the importance of using an insoluble solid-phase carrier having an average particle size of 0.01 to 1000 μm in a method as claimed in the above-identified application.

9. **Comparative Example 2: Results of using 5 nm polystyrene particles.**

10. In accordance with the same method as in Example 1 of the present application but using polystyrene particles having an average particle size of 5 nm instead of particles having an average particle size of 20 μn and having polystyrene surface and an iron oxide inner part, DNA was extracted and eluted from the solid carrier. Then, DNA and the solid carrier were separated by centrifugation at 15,000 r.p.m. for 1 hour. Measurement of absorbance showed an absorbance of about 0.2 at 320 nm, which revealed that there were minute particles which could not be separated. Continuous centrifugation for 1 hour under the same conditions decreased the absorbance at 320 nm to 0.17 but the absorbance did not become 0. Absorbance was measured from 320 nm to 240 nm under the resulting conditions. Due to the contamination of minute particles, the peak at around 260 nm, which is attributed to DNA, was not clear and the quantitative determination and purity determination of DNA was impossible. Accordingly, use of smaller size particles having an average particle size of

less than 0.01 μm made it difficult to separate particles and DNA, so that DNA having a high purity was not obtained.

11. **Comparative Example 3: Results of using 5 mm polystyrene particles**

12. In accordance with the same method as in Example 1 of the present application but using polystyrene particles having an average particle size of 5 mm instead of particles having an average particle size of 20 μm, DNA was extracted and eluted from the solid carrier. As shown in the table below, the amount of DNA recovered was remarkably decreased.

	Comparative Example 2	Comparative Example 3
Recovered DNA concentration (μg/ml)	Separation from the solid carrier was impossible, quantitative determination was impossible	40
Amount of recovered DNA (μg)	Separation from the solid carrier was impossible, quantitative determination was impossible	8
A260/A280	Separation from the solid carrier was impossible, quantitative determination was impossible	1.69
DNA Length	Not tested	150K
Hin dIII, *Eco* RI Digestion	Not tested	No trouble
Globulin PCR	Not tested	No trouble

13. Finally, there were typographical errors in Table 1 on page 33 of the application. The correct data are shown in the attached marked-up page.

14. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believe to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Kenjun Fan

Dr. Kenjun Fan

March. 5. 2007

Date

Table 1

	Recovered DNA concentration μg/ml	Amount of recovered DNA μg	A260/ A280	A260/ A230	DNA length	HindIII, EcoRI digestion	Globulin PCR	
Example 1	~~168~~ 16.8	34	1.95	2.03	150K	No trouble	No trouble	✓
Example 2	~~160~~ 16.0	32	1.98	2.01	150K	No trouble	No trouble	✓
Example 3	~~185~~ 18.5	37	2.05	2.06	150K	No trouble	No trouble	✓
Example 4	~~177~~ 17.7	35	2.04	1.99	150K	No trouble	No trouble	✓
Example 5	~~184~~ 18.4	37	1.98	1.97	150K	No trouble	No trouble	✓
Example 6	~~199~~ 19.9	40	1.99	1.99	150K	No trouble	No trouble	✓
Example 7	~~184~~ 18.4	37	2.08	2.04	150K	No trouble	No trouble	✓
Example 8	~~183~~ 18.3	(365) 36.5	1.97	1.98	150K	No trouble	No trouble	✓ ✓
Comparative Example 1	~~120~~ 12.0	24	1.99	2.01	150K	No trouble	No trouble	✓